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Changes in intestinal microbiota and abnormal amino acid metabolism lead to neurotransmitter disorders in patients with liver cirrhosis

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RESEARCH ARTICLE

ABSTRACT

The 16S rRNA sequencing technology was used to investigate changes in the abundance of intestinal microbiota, metabolites of blood and fecal samples were analyzed and their relationships with neurotransmitters were evaluated in patients with liver cirrhosis after hepatitis B infection. The liver function phenotypes correlated with Phylum Proteobacteria, Class Clostridia and Gamma Proteobacteria, Family Enterobacteriaceae, Ruminococcaceae, Streptococcaceae, Lachnospiraceae and Lactobacillaceae, Genus Faecalibacterium, Streptococcus, species *Escherichia coli*, etc. Genus Streptococcus has a good diagnostic value for patients with liver cirrhosis in the COM (Compensated liver disease) group, with an AUC of 0.81 (95% CI: 0.70–0.92), while Genus Streptococcus, Veillonella, Faecalibacterium, Blautia, and Bacteroides have a better diagnostic value for patients with liver cirrhosis in the DECOM (Decompensated liver disease) group (including DECOM1 and DECOM2), with the combined AUC reaching 0.93 (95% CI: 0.88–0.98). The level of ammonia in the DECOM2 group was significantly higher than that of the COM group ($P < 0.01$). Patients with post-hepatitis B cirrhosis have intestinal flora disorder, which leads to abnormal amino acid metabolism and further leads to neurotransmitter disorder in patients with cirrhosis and accelerates the disease progression. Probiotics can reduce the serum ammonia level in patients with cirrhosis and may prevent the occurrence of hepatic encephalopathy.

KEYWORDS

gut microbiota, liver cirrhosis, neurotransmitter disorder, hepatitis B, 16s rRNA sequencing

1. INTRODUCTION

Liver cirrhosis is a common clinical chronic progressive liver disease, which is diffuse liver damage formed by one or more causes for a long time or repeated actions [1]. A variety of liver and gallbladder diseases can lead to cirrhosis, such as viral hepatitis, drug-induced hepatitis, autoimmune hepatitis, fatty hepatitis, chronic inflammation and subsequent hepatocyte necrosis and fibrosis, diffuse bile duct obstruction, etc. [2]. Unlike alcoholic cirrhosis caused by excessive drinking and non-alcoholic cirrhosis caused by high-calorie food in Europe and the United States, chronic hepatitis B cirrhosis is the most common disease in Asia, especially in China [3, 4]. Hepatitis B virus replication leads to chronic liver inflammation and injury. During the period of liver injury, the dynamic balance between the deposition and degradation of extracellular matrix (ECM) is disrupted, leading to the activation of hepatic stellate cells (HSC) and the synthesis of a large number of ECM, which is the essence of liver fibrosis and the intermediate step in the development of liver cirrhosis [5]. In this study, the most common patients with post hepatitis B cirrhosis in China were selected as the research objects to investigate the changes of intestinal flora and metabolites in patients with post hepatitis B cirrhosis.

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Microbes in the human gut play an important role in health and disease, maintaining health and internal environment stability [6, 7]. Intestinal microbiota disturbances are common in patients with cirrhosis, and the severity of liver impairment is related to the extent of these disturbances, which are characterized by an increased abundance of potentially pathogenic bacteria and a decreased level of beneficial bacteria [8]. Some studies have shown that fecal transfer from patients with cirrhosis to sterile mice can lead to abnormal liver function in mice, suggesting that abnormal intestinal flora may be one of the inducements of cirrhosis [9]. Gut microbes, as the most complex and most abundant species in body organ systems, have the ability to release a variety of compounds [10]. The increase in the number of pathogens, the number of symbiotic bacteria reduce: An increase in the proportion of Bacteroides/Firmicutes, an increase in the number of pathogenic bacteria such as Enterobacteriaceae, and a decrease in the number of symbiotic bacteria such as Trichelicaceae can induce metabolic abnormalities in the human body [11]. At the same time, gut microbes can use the dietary components (such as carbohydrate proteins and fats) and host-derived components (including exhumed epithelial cells and mucus) of the host to power their own growth processes, and produce a variety of metabolites that affect the body's health and disease risk [12]. Existence of metabolic abnormalities in patients with cirrhosis, such as Hepatic encephalopathy (HE) is one of the end-stage liver diseases in the development of common metabolic nerve mental disorder syndrome with high morbidity and mortality. 60–80% of patients with decompensated cirrhosis will develop hepatic encephalopathy clinically, and the dominant HE prognosis is poor, with 1-year survival rates of 20–42% and 3-year survival rates of 15–23% [13]. A number of evidence have shown that the pathogenesis of HE is related to the increase of neural amino acids (pseudo neurotransmitters) such as aromatic amino acids (including phenylalanine, cucine and tryptophan) in the intestinal tract, the enhanced neural conduction mediated by inhibitory amino acid neurotransmitters GABA, and the weakened neural conduction mediated by excitatory amino acid neurotransmitters such as glycine, glutamate and aspartate [14, 15].

Although intestinal flora and metabolic abnormalities have been shown to be closely related to cirrhosis and hepatic encephalopathy, some questions remain unclear. For example: (1) there are many altered intestinal microbes in patients with cirrhosis, but which microbes are associated with the progression of cirrhosis? (2) What metabolic pathways are changed in patients with cirrhosis and what are the connections between intestinal microorganisms and neurotransmitters? This study explored the above issues, analyzed the characteristic intestinal microorganisms and metabolic pathways of patients with cirrhosis, and the relationship between them and the phenotypes and neurotransmitters of major patients with cirrhosis, such as ammonia, platelets, direct bilirubin, etc. to further explore the pathogenesis of cirrhosis. This study provides a basis for exploring the role of *Saccharomyces boulardii* in the treatment of cirrhosis.

2. MATERIALS AND METHODS

2.1. Case choice

A total of 22 patients with compensated liver cirrhosis post hepatitis B (COM group) and 26 patients with decompensated liver cirrhosis after hepatitis B (DECOM group) diagnosed in The Third Affiliated Hospital of Xinxiang Medical University from January 2019 to December 2019 were selected for the Child-Pugh score, and 21 healthy volunteers were selected as the NORMAL group. Another selection between January 2020 and August 2020 after the diagnosis of hepatitis B patients with cirrhosis of the liver decompensation period in 16 cases, 6 cases as a routine treatment group (No. 1–6, conventional group), given conventional treatment (including glutathione, Octreotide, furosemide, spironolactone and other medicine to protect liver function, reduce portal vein pressure, diuretic therapy, patients with high bilirubin received adenosylmethionine intravenously, patients with high serum ammonia received aspartate ornithine, and patients with low protein received human albumin intravenously); 10 patients in the *S. boulardii* group (No. 7–16, *S. boulardii* group) were given *S. boulardii* (Biocodex, France, registration number: S20150051, batch number: 3393), 0.5 g, twice a day, in addition to the conventional treatment group. Patients in both groups were given continuous medication for 28 days. If the hospitalization time did not reach 28 days, intravenous infusion drugs were changed to oral dosage form tablets for further consolidation therapy until the full course of treatment.

2.2. Diagnostic criteria

Patients included in this study had a confirmed history of chronic hepatitis B, meeting the diagnostic criteria for either compensatory or decompensatory stages of post-hepatitis B cirrhosis as outlined in the 2015 edition of the Guidelines for Prevention and Treatment of Chronic Hepatitis B. Exclusion criteria encompassed other etiologies of cirrhosis, such as alcoholic hepatitis, non-alcoholic steatohepatitis, autoimmune liver disease, genetic metabolic liver disease, schistosomiasis, and drug-induced hepatitis. Furthermore, all included participants exhibited evidence of ascites via B-ultrasound or CT imaging or presented with esophageal and gastric fundus varices confirmed by endoscopic or esophageal barium swallowing X-ray examination.

2.3. Inclusion criteria and exclusion criteria

Patients were included in this study if they were adult males or females aged 18–70 years, met the specified diagnostic criteria, had not received other clinical treatment within one month prior to enrollment, and were willing to participate, sign informed consent, and cooperate with the treatment protocol. Exclusion criteria comprised patients with recent concurrent hepatic encephalopathy, gastrointestinal massive hemorrhage, active infection, or liver cancer. Additionally, individuals with fatty liver disease, hypertension, diabetes, or

other systemic diseases were excluded, as were those who had used antibiotics, microbiological living agents, or lactulose within four weeks before sampling. Patients with complicated primary peritonitis, alcoholic hepatitis, non-alcoholic steatohepatitis, autoimmune liver disease, genetic metabolic liver disease, schistosomiasis, or drug-induced hepatitis, or other causes of cirrhosis, were also excluded from the study.

2.4. Genomic DNA amplification

500 mg of midcourse feces were taken from patients and healthy volunteers. Total RNA was extracted by the fecal genome extraction kit (Beijing Tiangen Biochemistry Technology Co., Ltd.) and the variable region of bacterial 16S rRNA gene V4 was amplified by PCR. High-fidelity PCR premixture (New England Biolabs, Inc.) of Phusion®GC buffer solution and high-efficient high-fidelity enzymes were used. The V4 variable region of bacterial 16S rRNA gene was amplified by PCR using barcode primers 514F ('-GTGCCAGCMGCCGCGGTAA-3′) and 805R (5′-GG ACTACHVGGGTWTCTAAT-3′). The amplification procedure was pre-denaturation at 94 °C for 2 min, denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, extension at 72 °C for 45 s, 30 cycles, and extension at 72 °C for 5 min. Each 25 μL PCR reaction system consisted of 0.5 μL template DNA, 2.0 μL dNTP mixture (2.5 mM, TaKaRa), 2.5 μL non-10 × Mg^2 + Ex Taq buffer, 1.5 μL Mg^2 + (25 mM), 0.25 μL Lex Taq DNA polymerase (2.5 units), 0.5 μL 10 mol L^{-1} primer 514F, 0.5 μL 10 mol L^{-1} primer 805R, and 17.25 μL double stewed water. Sterile double steamed water was used for dilution. The library was constructed using TruSeq® DNA PCR-Free Sample Preparation Kit, and the variable region of bacterial rRNA gene V4 was sequenced using HiSeq2500 PE250 platform [16].

2.5. Laboratory examination

Fasting venous blood was extracted from the subjects and centrifugated at 4000 r/min at 4 °C for 10 min. Serum was collected for ALT, TIBL and ALB detection, these were measured by the biochemical method adopted by Hitachi 7180 series fully automatic biochemical analyzer test, the prothrombin time (PT) was measured by Japan Sysmex prothrombin analyzer test, blood ammonia (AMM) was measured by the American VITROS 350 biochemical analyzer test. Venous blood was extracted, and platelet (PLT) was detected by Sysmex XN-10 hematology analyzer from Japan.

2.6. Statistical analysis

SPSS21.0 software was used for statistical analysis. Measurement data was expressed as $x \pm s$, and the general measurement data was tested by one-way ANOVA. R software (version 2.15.3) was used for principal coordinate analysis (PCOA). Pearson method was used for correlation analysis ($r > |0.3|$, $P < 0.05$ for correlation). Tax4Fun software package and MATLAB software were used to construct Thiessen polygon for function prediction of intestinal flora [17]. Variable Importance in the Projection (VIP) of the first principal component of the PLS-DA model was used to search for differentially expressed metabolites in combination with the *P*-value of *T*-test. The differentially expressed metabolites were screened out by setting the threshold of VIP > 2.0, the differentially expressed multiple of FC BBB > 0 or FC < 0.5 and *P* value < 0.05. In mice, the gene ontology (GO) information of the altered expression protein was analyzed using David software (Release 6.8). Classified annotations are provided in GO and KEGG forms. The difference significance threshold was set as follows: enrichment ≥2.0, multiple enrichment ≥2.0, Benjamini correction ($P < 0.05$). Cytoscape software (version 3.3.0) was used to build the network. ROC analysis was performed with GraphPad Prism 6.0. $P < 0.05$ was considered statistically significant.

3. RESULTS

3.1. Demographic and clinical characteristics of study

The study cohort was stratified into five phenotypic categories: NORMAL, COM (compensated), DECOM (decompensated), DECOM1, and DECOM2, with a male predominance observed across all groups as shown in Table 1. The mean age of participants was comparable

Table 1. Clinical parameters of patients in this study

Phenotypic	NORMAL	COM	DECOM	DECOM1	DECOM2
Gender	Male = 15 Female = 6	Male = 15 Female = 7	Male = 18 Female = 8	Male = 9 Female = 6	Male = 9 Female = 2
Age	51.6 ± 7.3	47.9 ± 9.0	51.6 ± 10.5	52.3 ± 11.1	50.5 ± 10.1
Child-Pugh	5 ± 0.0	5.318 ± 0.6463	9.077 ± 2.261	9 ± 2.171	9.182 ± 2.483
ALT(U/L)	19.71 ± 9.587	79.86 ± 100.5	62.88 ± 71.45	80.67 ± 85.23	38.64 ± 38.36
TBIL (μM)	12.86 ± 4.575	21.82 ± 9.97	52.31 ± 38.25	45.27 ± 30.77	61.91 ± 46.43
ALB (g L^{-1})	45.33 ± 2.497	42.23 ± 6.301	32.5 ± 5.887	33.07 ± 7.086	31.73 ± 3.901
PT (s)	11.38 ± 0.669	14.59 ± 1.469	17.92 ± 2.827	17.87 ± 2.9	18 ± 2.864
PLT (10^9/L)	242.7 ± 60.23	94.55 ± 46.1	78.35 ± 47.99	89.2 ± 58.65	63.55 ± 22.89
AMM (μM)	5.771 ± 1.059	23.12 ± 15.03	32.63 ± 16.06	29.84 ± 13.75	36.45 ± 18.77

NORMAL: Control group without liver disease; COM: Compensated liver disease; DECOM: Decompensated liver disease; DECOM1: Decompensated liver disease subgroup 1; DECOM2: Decompensated liver disease subgroup 2; ALT: Alanine Aminotransferase (U/L); TBIL: Total Bilirubin (μM); ALB: Albumin (g L^{-1}); PT: Prothrombin Time (s); PLT: Platelet Count (10^9/L); AMM: ammonia (μM).

among groups, ranging from 47.9 ± 9.0 years (COM) to 52.3 ± 11.1 years (DECOM1), suggesting no significant age-related differences in disease progression. Liver disease severity, as assessed by the Child-Pugh score, exhibited a marked increase in decompensated groups (DECOM: 9.077 ± 2.261, DECOM1: 9 ± 2.171, DECOM2: 9.182 ± 2.483) compared to compensated (COM: 5.318 ± 0.6463) and normal (5 ± 0.0) groups, confirming progressive hepatic dysfunction.

3.2. Biochemical and hematological markers of liver function

Liver injury, indicated by ALT levels, was notably elevated in COM (79.86 ± 100.5 U/L) and DECOM1 (80.67 ± 85.23 U/L), suggesting active hepatocellular damage, whereas DECOM2 (38.64 ± 38.36 U/L) displayed lower levels, potentially reflecting diminished hepatocyte mass in advanced disease as shown in Table 1. Total bilirubin (TBIL) exhibited a progressive rise from NORMAL (12.86 ± 4.575 μM) to DECOM2 (61.91 ± 46.43 μM), consistent with worsening hepatic excretory capacity. Similarly, albumin (ALB) levels declined significantly in decompensated groups (DECOM: 32.5 ± 5.887 g L^{-1}, DECOM2: 31.73 ± 3.901 g L^{-1}) compared to NORMAL (45.33 ± 2.497 g L^{-1}), indicative of impaired synthetic function. Prolonged prothrombin time (PT) in decompensated cirrhosis (DECOM: 17.92 ± 2.827 s, DECOM2: 18 ± 2.864 s) versus NORMAL (11.38 ± 0.669 s) further corroborated coagulopathy due to deficient clotting factor production.

3.3. Hematological and metabolic alterations

Platelet counts (PLT) were substantially reduced in decompensated groups (DECOM: 78.35 ± 47.99 × 10^3/μL, DECOM2: 63.55 ± 22.89 × 10^3/μL) relative to NORMAL (242.7 ± 60.23 × 10^3/μL), likely secondary to portal hypertension and splenic sequestration as shown in Table 1. Ammonia (AMM) levels were significantly elevated in COM (23.12 ± 15.03 μM) and further increased in DECOM (32.63 ± 16.06 μM) and DECOM2 (36.45 ± 18.77 μM), aligning with the progression of hepatic encephalopathy in advanced liver disease. These findings demonstrate a clear phenotypic gradient in liver disease severity, with progressive deterioration in hepatic synthetic, excretory, and metabolic functions across groups.

3.4. Gut microbiota correlations with liver disease markers

The analysis of the gut microbiota and clinical liver markers established a distinct microbial signature strongly correlated with liver disease severity. Taxa belonging to the Firmicutes phylum demonstrated a protective profile, particularly Clostridia (class) and its related families, showing significant negative associations with markers of hepatic dysfunction. Notably, *Clostridia* exhibited a powerful inverse correlation with the Child-Pugh score ($\rho = -0.7054$, $P < 0.05$) and Total Bilirubin (TIBL) ($\rho = -0.5884$, $P < 0.05$), coupled with a strong positive correlation with the beneficial marker Albumin (ALB) ($\rho = 0.6624$, $P < 0.05$). Conversely, taxa implicated in disease worsening, primarily the *Proteobacteria phylum*, displayed strong positive correlations with severity indicators. The overall phylum was positively associated with TIBL ($\rho = 0.4270$, $P < 0.05$) and negatively associated with ALB ($\rho = -0.4109$, $P < 0.05$). Furthermore, members of the *Bacilli* class, including the genus *Streptococcus*, showed a potent deleterious influence, with *Streptococcus* correlating positively with the Child-Pugh score ($\rho = 0.6687$, $P < 0.05$) and negatively with ALB ($\rho = -0.4628$, $P < 0.05$). These data indicate that advanced liver disease is characterized by a significant depletion of beneficial Clostridiales and a concomitant enrichment of harmful *Proteobacteria* and *Bacilli*, highlighting key targets for therapeutic modulation (Table 2).

4. DISCUSSION

The liver interacts directly with the intestine through the hilum and bile secretion system. Liver dysfunction has a number of effects on the gut, including impaired movement and reduced bile flow, which can lead to dysregulation of the gut microbiome [18, 19]. Bile acids have direct bacteriostasis. Liver dysfunction can lead to a decrease in the proportion of secondary bile acids and primary bile acids, a decrease in bile flow and intestinal bile acids in patients with cirrhosis, which affects the composition of intestinal flora and ultimately leads to the excessive growth of intestinal pathogenic bacteria [20]. In this study, it was found that patients with cirrhosis had dysregulation of intestinal flora, such as decreased α diversity and increased overall differences (β diversity) between groups. Through LEFSE analysis, we have obtained biomarkers such as genus *Lactobacillus, Subdoligranulum, Ruminococcaceae, Blautia, Roseburia, Veillonella, Streptococcus, species Staphylococcus salivarius subsp. thermophilus* in phylum firmicutes, species *Escherichia coli* in phylum proteobacteria. These biomarkers are associated with Child-Pugh, ALT, TIBL, ALB, PT, PLT and AMM phenotypes. Among the Firmicutes, *Lactobacillus, Subdoligranulum, Ruminococcaceae, Blautia* and *Roseburia* can promote the growth of butyric acid producing bacteria and maintain intestinal integrity [21]. *Subdoligranulum, Ruminococcaceae* and *Lactobacillus* are all negatively correlated with child-pugh and AMM. The decrease of *Subdoligranulum, Ruminococcaceae* and *Lactobacillus* can lead to the decrease of lactic acid production, the increase of intestinal pH and the decrease of ammonia excretion and thus induce or aggravate hepatic encephalopathy [22]. Increasing pH can also lead to microbial involvement in amino acid fermentation, which can produce branched chain fatty acids (BCFA), SCFA and potentially harmful metabolites [23, 24]. Elevated *Veillonella and Streptococcus* in Firmicutes have been proved to be related to cirrhosis. Their excessive growth can decompose amino acids, purines and urea in the intestine, resulting in elevated blood ammonia and further aggravating liver function damage

Table 2. Intestinal microbiota and correlation with Child-Pugh, ALT, TIBL, ALB, PT, PLT and AMM values in patients of this study

		Child-Pugh	ALT	TIBL	ALB	PT	PLT	AMM
Phylum								
	Firmicutes	−0.1802	0.0637	−0.2601*	0.2937*	−0.1730	0.3013*	−0.2737*
	Proteobacteria	0.3602*	−0.0930	0.4270*	−0.4109*	0.3679*	−0.3526*	0.3469*
	Actinobacteria	0.145	−0.158	0.191	−0.1740	0.003	0.057	−0.195
Class								
	Clostridia	−0.7054*	−0.0762*	−0.5884*	0.6624*	−0.592*	0.4943*	−0.4703*
	Gamma proteobacteria	0.3733*	−0.0860	0.4399*	−0.4291*	0.3825*	−0.3659*	0.3590*
	Negativicutes	0.2182	0.0038	0.1415	−0.4008*	0.2138	−0.2294	0.2751*
	Bacilli	0.6697*	0.1754	0.4495*	−0.3888*	0.5219*	−0.2287	0.2103
	unidentified_Actinobacteria	−0.0757	0.1490	−0.0725	0.1608	−0.1672	0.1171	−0.0595
Order								
	Clostridiales	−0.7054*	−0.0762	−0.5884*	0.6624*	−0.5920*	0.4943*	−0.4703*
	Enterobacteriales	0.3524	−0.0897	0.3933*	−0.4011*	0.3834*	−0.3525*	0.3393*
	Selenomonadales	0.2182	0.0038	0.1415	−0.4008*	0.2138	−0.2294	0.2751*
	Lactobacillales	0.6706*	0.1758	0.4504*	−0.3896*	0.5231*	−0.2296	0.2117
	Bacteroidales	−0.1954	−0.0684	−0.2012	0.0439	−0.1721	−0.0084	−0.1187
	Bifidobacteriales	0.150	−0.147	0.195	−0.173	0.003	0.055	−0.181
Family								
	Enterobacteriaceae	0.3524	−0.0897	0.3933*	−0.4011*	0.3834*	−0.3525*	0.3393*
	Ruminococcaceae	−0.4893*	−0.0528	−0.4493*	0.4346*	−0.4193*	0.3734*	−0.4199*
	Veillonellaceae	0.2246	0.0076	0.1486	−0.4084*	0.2233	−0.2337	0.2770*
	Streptococcaceae	0.6633*	0.2068	0.4210*	−0.4576*	0.4991*	−0.2597*	0.2357
	Lachnospiraceae	−0.5893*	−0.0576	−0.4644*	0.5717*	−0.4853*	0.3470*	−0.2981*
	Bifidobacteriaceae	−0.0737	0.1519	−0.0651	0.1484	−0.1506	0.1060	−0.0448
	Lactobacillaceae	0.3190*	−0.0248	0.2449*	−0.0845	0.2872*	−0.0704	0.0621
	Bacteroidaceae	−0.1954	−0.0684	−0.2012	0.0439	−0.1721	−0.0084	−0.1187
Genus								
	Faecalibacterium	−0.3325*	−0.0784	−0.3069*	0.2532*	−0.2575*	0.1251	−0.2594*
	Megamonas	0.0393	0.0474	0.0404	−0.2769*	0.0980	−0.1809	0.3009*
	Streptococcus	0.6687*	0.2002	0.4238*	−0.4628*	0.5034*	−0.2659*	0.2389*
	unidentified_Enterobacteriaceae	0.3576*	−0.0708	0.1737	−0.2449*	0.3419*	−0.2793*	0.3002*
	Lactobacillus	0.3204*	−0.0248	0.2448*	−0.0845	0.2871*	−0.0705	0.0623
	Bifidobacterium	0.150	−0.146	0.195	−0.173	0.003	0.055	−0.181
	Blautia	0.231	−0.004	−0.103	−0.261	0.083	−0.030	−0.030
	Veillonella	0.4489*	0.0353	0.3305*	−0.3946*	0.3041*	−0.2502*	0.1286
	unidentified_Ruminococcaceae	−0.4735*	−0.1042	−0.3699*	0.4778*	−0.393*	0.3245*	−0.2845*
	unidentified_Lachnospiraceae	−0.4427*	−0.1353	−0.2685*	0.421*	−0.3463*	0.2842*	−0.1264
	Roseburia	−0.2188	0.0486	−0.1767	0.1763	−0.1452	−0.1154	−0.0227
	Bacteroides	−0.1954	−0.0684	−0.2012	0.0439	−0.1721	−0.0084	−0.1187
	Subdoligranulum	−0.3449*	0.0262	−0.2503*	0.3797*	−0.2997*	0.2299	−0.2719*
Species								
	Escherichia coli	0.3608*	−0.0693	0.1751	−0.2451*	0.3430*	−0.2781*	0.2994*
	Streptococcus salivarius subsp	−0.104	−0.208	0.246	0.021	0.268	−0.001	−0.196
	Lactobacillus salivarius	−0.3260	0.0000	−0.2810	0.2670	−0.2650	−0.0480	−0.0570
	Ruminococcus sp 5_1_39BFAA	−0.0615	−0.0025	−0.0883	0.1420	−0.1747	0.2319	0.0127
	Bifidobacterium adolescentis	−0.2140	−0.0787	−0.1585	0.2013	−0.1940	0.1964	−0.1342
	Staphylococcus salivarius subsp. *thermophilus*	0.5083*	0.1713	0.3026*	−0.3709*	0.4093*	−0.2041	0.3219*
	Bacteroides uniformis	−0.2119	−0.1286	−0.1947	0.2469*	−0.2855*	0.2131	−0.2243
	Roseburia inulinivorans	−0.0863	−0.0354	−0.0781	0.0032	−0.0241	−0.1978	−0.0263

*, $P < 0.05$, Child-Pugh: Child-Pugh score; ALT: Alanine Aminotransferase; TIBL: Total Bilirubin; ALB: Albumin; PT: Prothrombin Time; PLT: Platelet Count; AMM: Amount.

[25]. The relative abundance of *S. salivarius subsp. thermophilus* was also positively correlated with cirrhosis and the damage to liver function might be related to Staphyolysin and Enterotoxin. Proteobacteria is a biomarker of decompensated liver cirrhosis group, especially the more serious DECOM2 group. Therefore, we believe that such microorganisms may overmultiply with liver function injury and intestinal flora dysregulation, aggravating the disease of cirrhosis. For example, *E. coli* is a common inhabitant of human and animal intestines, which is harmless to human body under normal circumstances [26]. However, in this study, the relative abundance of *E. coli* was positively correlated with liver cirrhosis, which may be related to the disturbance of intestinal flora caused by liver function impairment in patients with liver cirrhosis, and then the excessive abundance of *E. coli* in the intestinal tract and its endotoxin further aggravating liver injury. We found that Genus Streptococcus is a good biomarker for the compensatory stage of liver cirrhosis, and Genus Streptococcus, *Veillonella, Faecalibacterium, Blautia* and Bacteroides are good biomarkers for the diagnosis of deco compensatory stage of liver cirrhosis.

Hepatic encephalopathy (HE) is a reversible metabolic disorder caused by central nervous system dysfunction in patients with acute or chronic liver disease [27]. Hepatic encephalopathy is still a common complication in patients with acute and chronic liver diseases, especially in patients with decompensated cirrhosis and liver failure [28]. The main manifestations of hepatic encephalopathy include abnormal changes in cognitive function, mood, behavior and motor function, and even neuropsychiatric disorder syndrome in coma [29]. The pathogenesis of hepatic encephalopathy is not yet clear. Although the theory of ammonia poisoning has always been dominant in the pathogenesis hypothesis, clinically, the degree of hepatic encephalopathy is not parallel to the concentration of arterial blood ammonia, and high blood ammonia itself does not appear the EEG manifestation of hepatic encephalopathy. Therefore, the abnormal metabolism of amino acids, the formation of pseudo neurotransmitters and the dominant inhibitory neurotransmitters of amino acids are also important causes of hepatic encephalopathy. In addition, pseudo neurotransmitters and inhibitory amino acid neurotransmitters are also caused by abnormal amino acid metabolism, which is associated with ammonia poisoning to some extent [30]. Aroma amino acids (phenylalanine, tyrosine, tryptophan, etc.) and GABA mainly come from the gut. Intestinal microbial dysregulation alters normal amino acid metabolism [31]. In order to further confirm the influence of intestinal flora, especially potential biomarkers, on amino acid metabolism, we investigated the changes of metabolites in the feces of patients with cirrhosis through metabolomics, and KEGG analysis confirmed that the feces of patients with cirrhosis showed significant metabolic abnormalities, such as energy metabolism, fatty acids, amino acids, bile acid, vitamin and other metabolic disorders. Purine metabolism levels were significantly different between COM and DECOM1 groups, which may play an important role in the progression from compensated cirrhosis to mildly decompensated cirrhosis. Glycine is not only a constituent amino acid of endogenous antioxidant reduced glutathione, but also an excitatory neurotransmitter. In this study, the glycine of the DECOM1 group was significantly lower than that of the COM group.

The levels of phenylalanine metabolism and purine metabolism were significantly different between DECOM1 and DECOM2 groups, which may be an important mechanism for the progression of decompensated cirrhosis. N-acetyl-L-phenylalanine is an important intermediate product in the synthesis of the important pseudo neurotransmitter L-phenylalanine and is a component of phenylalanine metabolism. In this study, N-acetyl-L-phenylalanine was significantly increased in the DECOM2 group compared with the DECOM1 group, and microorganisms may regulate the level of L-phenylalanine by affecting the metabolism of phenylalanine. With the progression of liver disease, the clearance of tyrosine of aromatic amino acids and phenylalanine metabolites tyramine and phenylethylamine is affected, and the levels of these two amines increase can penetrate the blood-brain barrier into the brain tissue and can be catalyzed by p-hydroxylase to form p-hydroxytyramine and phenylpropanolamine respectively. Although the latter are chemically similar to the excitatory neurotransmitter norepinephrine, they do not transmit nerve impulses or are less excitatory. Therefore, if pseudo neurotransmitters are ingested by brain cells and replace the normal excitatory neurotransmitters in synapses, nerve conduction will be inhibited, and then the symptoms of hepatic encephalopathy such as memory decline, confusion, lack of energy, coma, etc., it may be one of the causes of hepatic encephalopathy in patients (Carnagarin 2021). These results confirmed the influence of intestinal flora on amino acid and purine metabolism pathways in KEGG analysis. It can also be seen from the prediction of bacterial flora phenotype that the amino acid synthesis and metabolic phenotype of intestinal flora of patients with cirrhosis increase with the exacerbation of the disease, which proves that intestinal flora may cause the increase of blood ammonia by affecting the amino acid and purine metabolism pathways.

S. boulardii is a fungal agent, belongs to the probiotics, uniform composition, good stability, it can activate the complement system and reticuloendothelial system and increase the endogenous defensive barrier, preventing the invasion and reproduction of pathogenic bacteria, reducing bacterial translocation [32]. This is conducive to maintaining intestinal microecological balance and reducing intestinal mucosal cell damage to alleviate the clinical symptoms of patients. In this study, the conventional treatment group was provided liver preservation [33], yellow-removal, diuresis, portal venous pressure reduction and symptomatic supportive treatment for patients with decompensated cirrhosis, *S. boulardii* group based on conventional treatment method using *S. boulardii*, we found in the two groups after treatment of ALT, TIBL, ALB, AMM, Child-Pugh score was significantly improved than before treatment, We considered that it was mainly related to the combined treatment of

liver protection, yellow-removal and protein-input support. There was no significant improvement in PT and PLT in both groups after treatment. Considering that the changes in PLT were mainly related to hypersplenism in patients with cirrhosis, a short period of treatment would not cause any improvement in PLT. Short-time improvement of PT is often associated with clinical infusion of fresh plasma, but in general, patients with PT greater than 20 s are recommended for clinical infusion of plasma, and most of the enrolled patients in this study did not meet the standard of clinical infusion of plasma.

After treatment, there were no significant differences in Child-Pugh, ALT, TIBL, ALB, PT and PLT between the *S. troulardii* group and the conventional treatment group. Considering that *S. troulardii* is a kind of probiotic, the therapeutic effect of *S. troulardii* is mainly reflected in the regulation of intestinal flora, and the short-term treatment still has little effect in improving the overall condition of patients with cirrhosis. However, the AMM level of more patient in *S. Bulardii* group was decreased than that of the conventional treatment group, and it had certain regulation effects on intestinal microorganisms such as Megamona, Bautia, Unidentified_lachnospiraceae and Unidentified Ruminococcaceae, which was related to the fact that *S. Bulardii* could inhibit the significant overgrowth of bacteria such as *Enterobacteriaceae*, reduce the production of endotoxin, improve the intestinal barrier function, reduce ammonia absorption and blood ammonia level. It may play a role in reducing the occurrence of HE.

5. CONCLUSION

This study investigated the influence of intestinal microbial-liver metabolic network on the progression of cirrhosis, provided a new research idea for the pathogenesis of cirrhosis, and verified the hypothesis that intestinal flora induces HE through metabolic pathway. Through screening metabolites and microorganisms related to disease stage, we explore the new strategy of diagnosis and prognosis of cirrhosis. In future studies, we will further explore the HE treatment targeting intestinal bacteria. We believe that the improvement of intestinal microbiota and metabolite abnormalities may contribute to the reduction of liver injury and cirrhosis related complications, which will become a new idea for the prevention and treatment of HE patients.

Availability of data and materials: All data generated or analyzed during this study are included in this published article.

Authors' contributions: XG and KZ was responsible for the conception and design of the study. SL was responsible for data collection and interpretation. All authors read and approved the final manuscript.

Conflicts of interest: The remaining authors have no conflicts of interest to report.

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